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(54) **METHOD AND SYSTEM FOR FULL-FIELD INTERFERENCE MICROSCOPY IMAGING**

(71) Applicant: **LLTECH MANAGEMENT**, Paris (FR)

(72) Inventors: **Albert Claude Boccara**, Paris (FR);  
**Fabrice Harms**, Orsay (FR)

(73) Assignee: **LLTECH MANAGEMENT**, Paris (FR)

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(56) **References Cited**

U.S. PATENT DOCUMENTS

5,459,564 A \* 10/1995 Chivers ..... G01B 9/04  
356/477  
6,480,285 B1 \* 11/2002 Hill ..... G01B 9/04  
356/492

(Continued)

*Primary Examiner* — William R Alexander

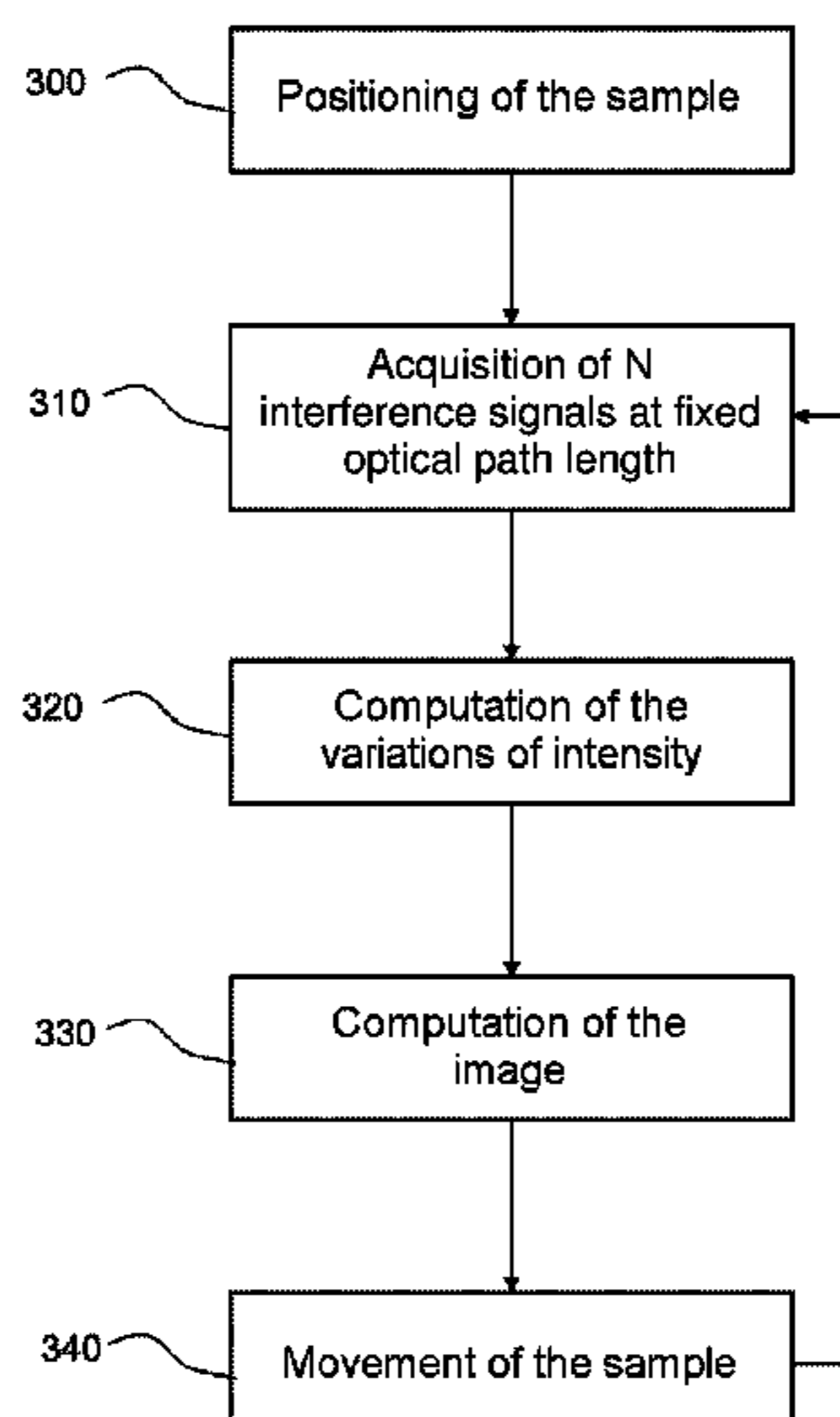
*Assistant Examiner* — Balram T Parbadia

(74) *Attorney, Agent, or Firm* — Osha Liang LLP

(57) **ABSTRACT**

The invention relates to a system (20) for full-field interference microscopy imaging of a three-dimensional diffusing sample (206). Said system includes: —an interference device (200) including a reference arm on which a reflective surface (205) is arranged, the interference device being suitable for producing, at each point of an imaging field when the sample is placed on a target arm of the interference device, interference between a reference wave, obtained by reflection of incident light waves onto a basic surface of the reflective surface (205) corresponding to said point of the imaging field, and a target wave obtained by backscattering of incident light waves by means of a voxel of a slice of the sample at a given depth, said voxel corresponding to said point of the imaging field; —an acquisition device (208) suitable for acquiring, at a fixed path length difference between the target arm and the reference arm, a temporal series of N two-dimensional interferometric signals resulting from the interference produced at each point of the imaging field; and —a processing unit (220) configured to calculate an image (IB, IC) representing temporal variations in intensity between said N two-dimensional interferometric signals.

**10 Claims, 8 Drawing Sheets**

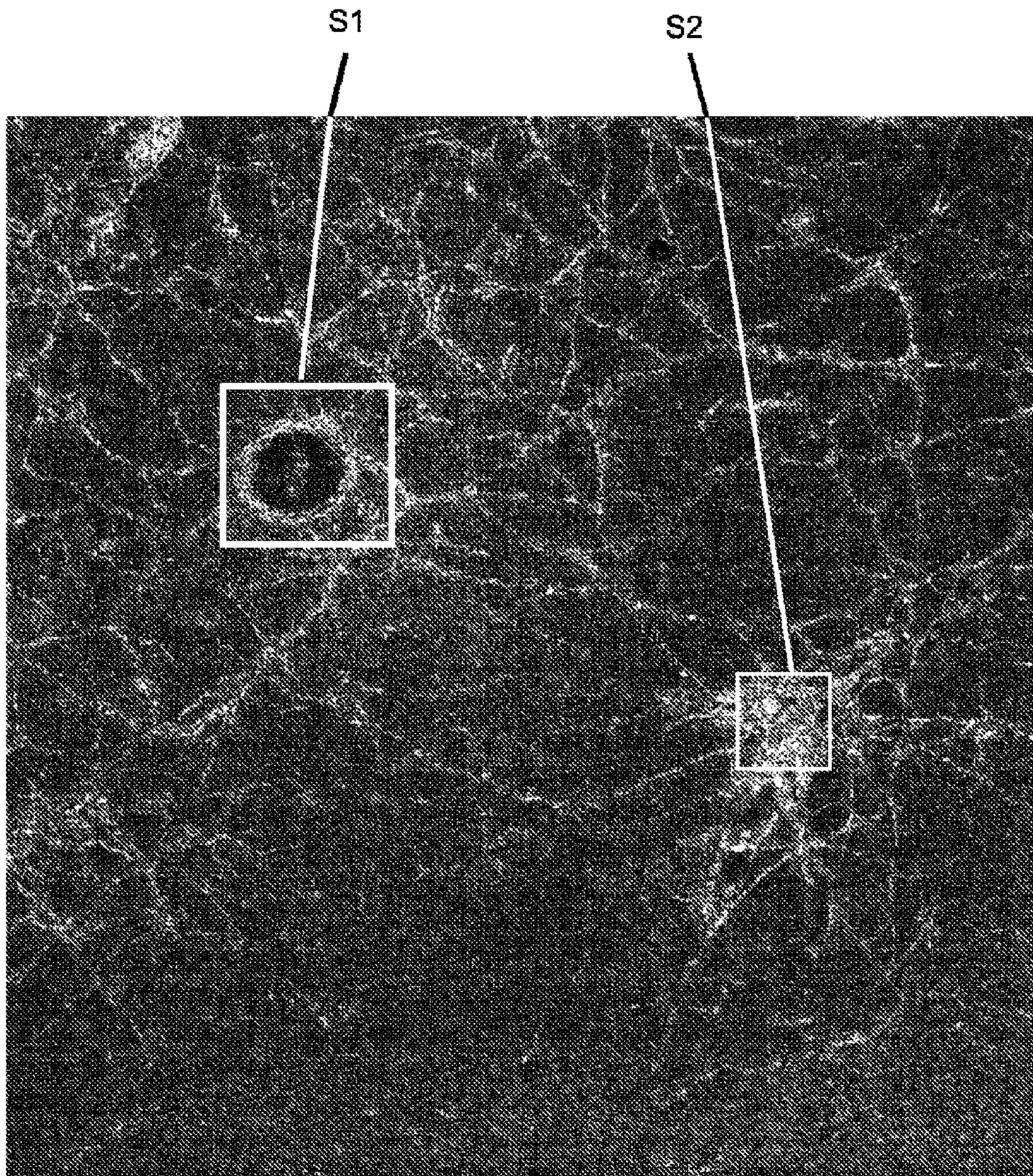


**US 10,627,613 B2**

(51)	<b>Int. Cl.</b>		7,034,271 B1 *	4/2006	Sinclair .....	G02B 21/0016 250/201.3
	<i>G01B 9/02</i>	(2006.01)				
	<i>G02B 21/00</i>	(2006.01)	7,623,908 B2 *	11/2009	Boppart .....	G01J 3/44 600/477
	<i>G01N 21/47</i>	(2006.01)				
	<i>G02B 21/08</i>	(2006.01)	7,768,651 B2 *	8/2010	Ueno .....	A61B 3/102 356/497
	<i>G06T 5/50</i>	(2006.01)				
	<i>G02B 21/18</i>	(2006.01)	8,054,468 B2 *	11/2011	de Boer .....	A61B 5/0059 356/456
	<i>G06T 1/00</i>	(2006.01)				
			2004/0105100 A1 *	6/2004	Shirley .....	G01B 11/2527 356/603
(52)	<b>U.S. Cl.</b>					
	CPC .....	<i>G01N 21/4795</i> (2013.01); <i>G02B 21/0004</i> (2013.01); <i>G02B 21/082</i> (2013.01); <i>G02B</i> <i>21/14</i> (2013.01); <i>G06T 5/50</i> (2013.01); <i>G01N</i> <i>2021/4709</i> (2013.01); <i>G02B 21/18</i> (2013.01); <i>G06T 1/0007</i> (2013.01); <i>G06T 2207/10101</i> (2013.01)	2005/0225769 A1 *	10/2005	Bankhead .....	G01B 11/2441 356/497
			2007/0237445 A1 *	10/2007	Hatori .....	G01N 21/4795 385/11
			2008/0304144 A1 *	12/2008	Reimer .....	A61B 5/0066 359/377
			2013/0107268 A1 *	5/2013	Boccara .....	G01B 9/02 356/450
(58)	<b>Field of Classification Search</b>					
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	See application file for complete search history.		2013/0148106 A1 *	6/2013	Tearney .....	A61B 5/0062 356/72
			2013/0182096 A1 *	7/2013	Boccara .....	A61B 5/0066 348/79
(56)	<b>References Cited</b>					
	U.S. PATENT DOCUMENTS		2014/0375792 A1 *	12/2014	Yaqoob .....	G02B 21/14 348/79
	6,721,094 B1 *	4/2004	Sinclair .....			G01B 9/04 356/445

\* cited by examiner





IA

PRIOR ART

FIG.1



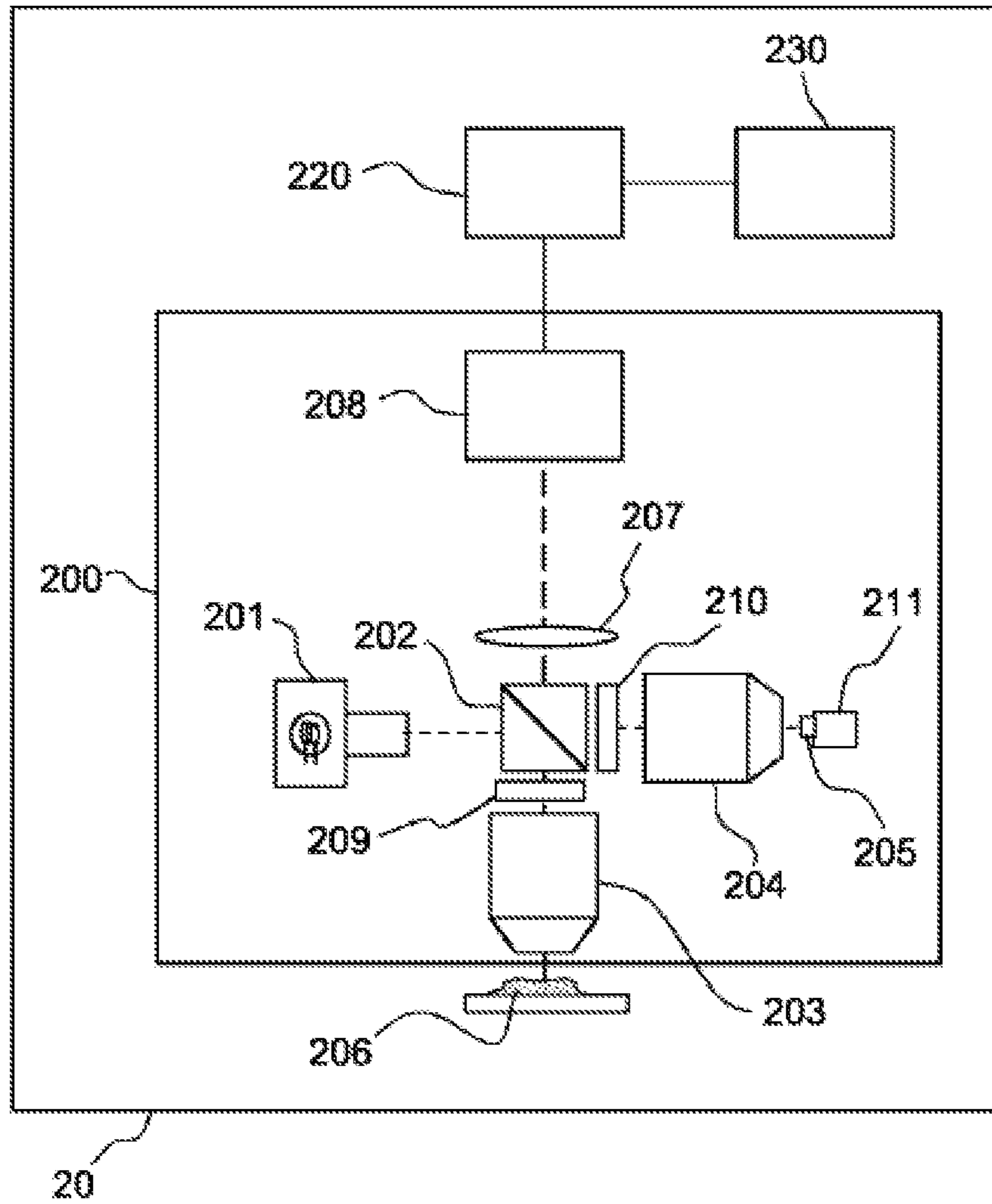


FIG.2

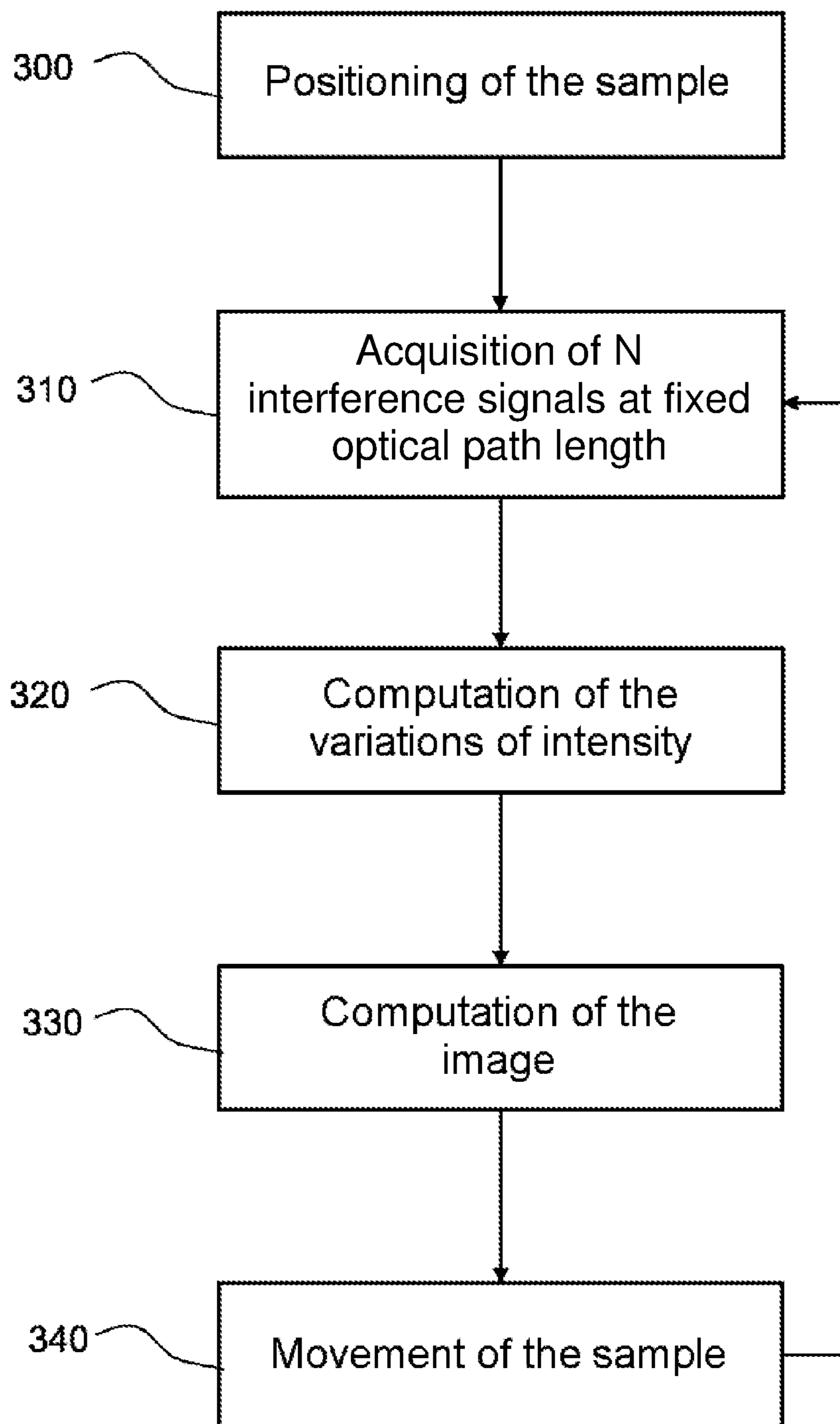


FIG.3



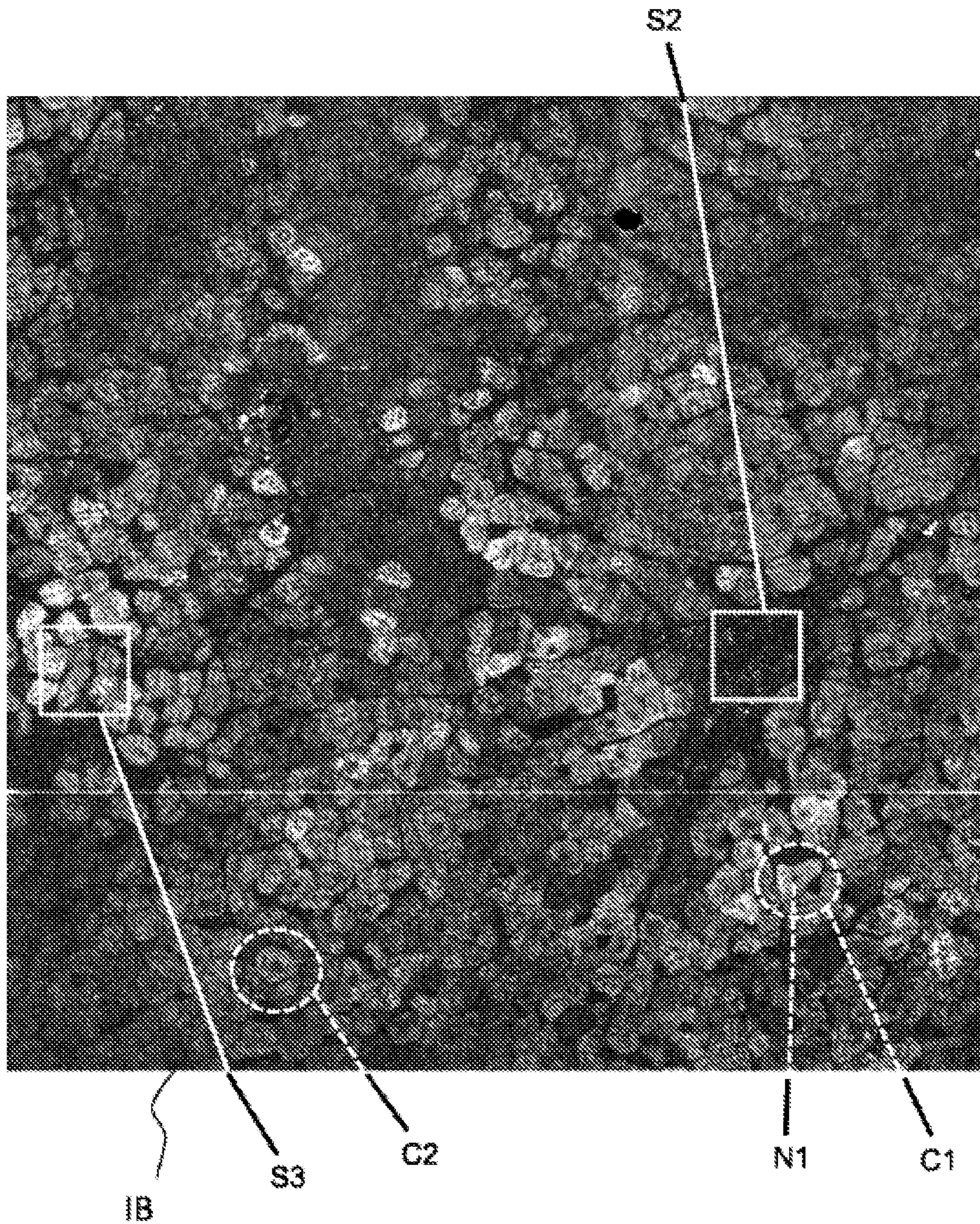


FIG.4



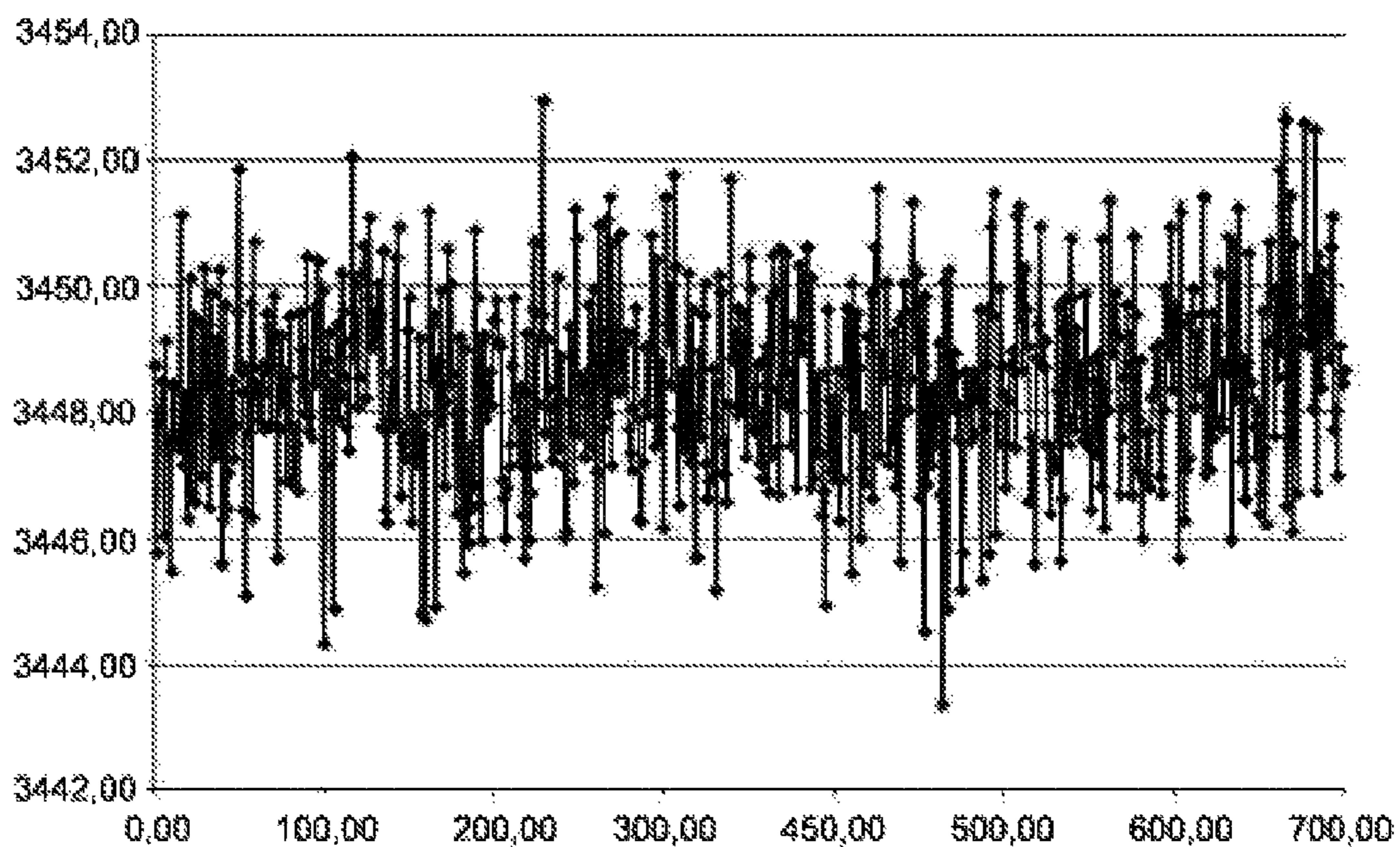


FIG.5A

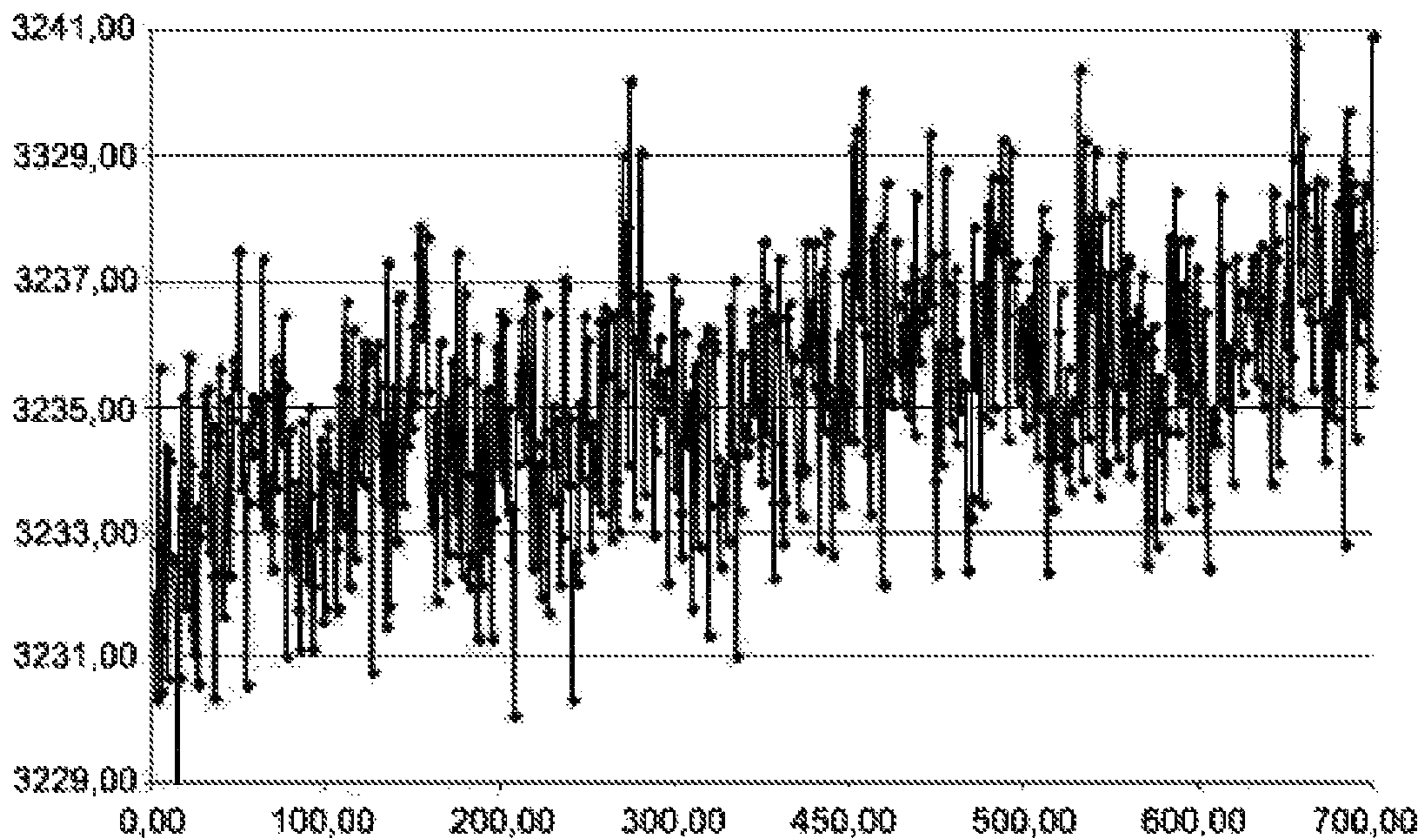


FIG.5B



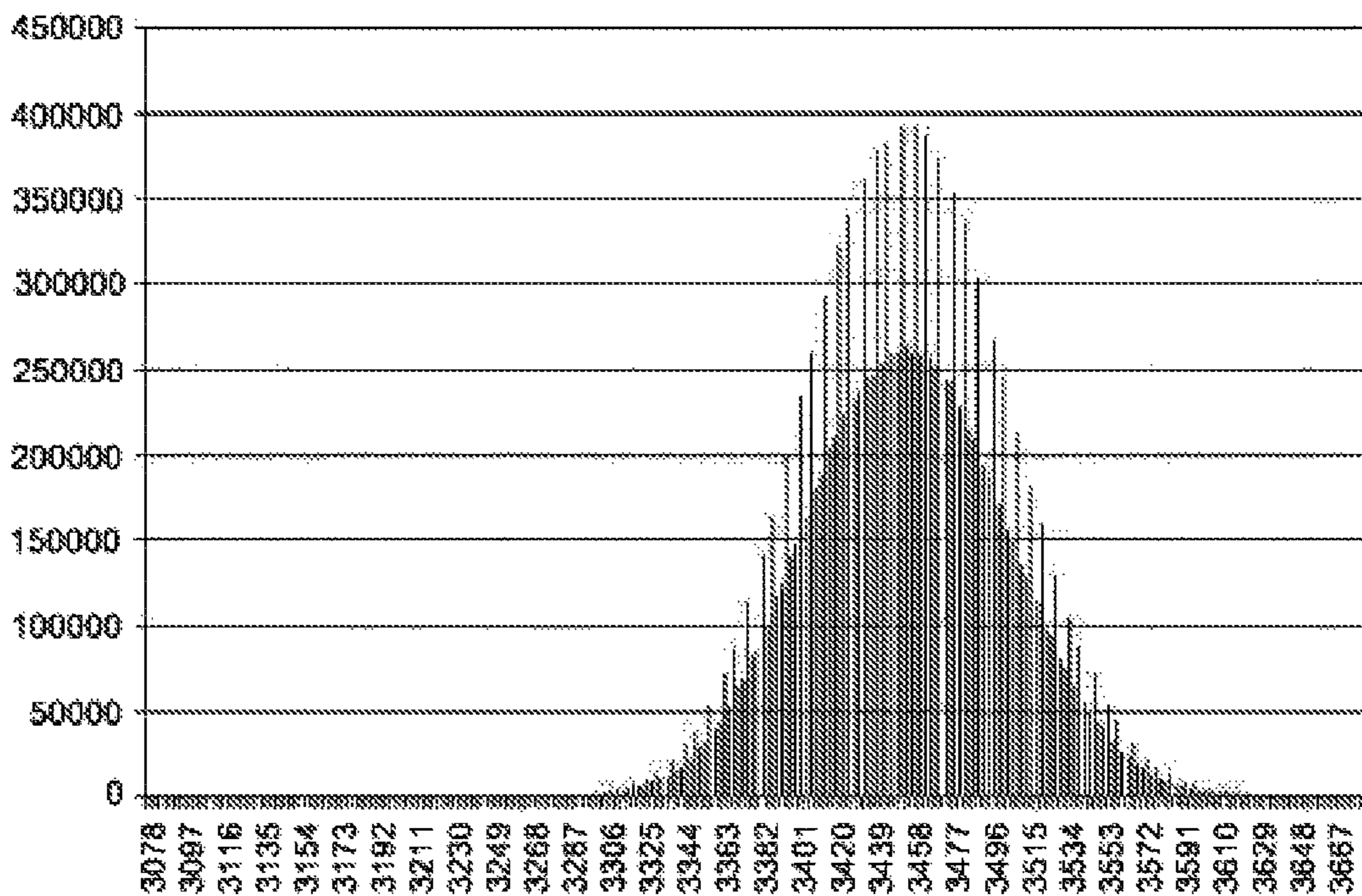


FIG. 6A

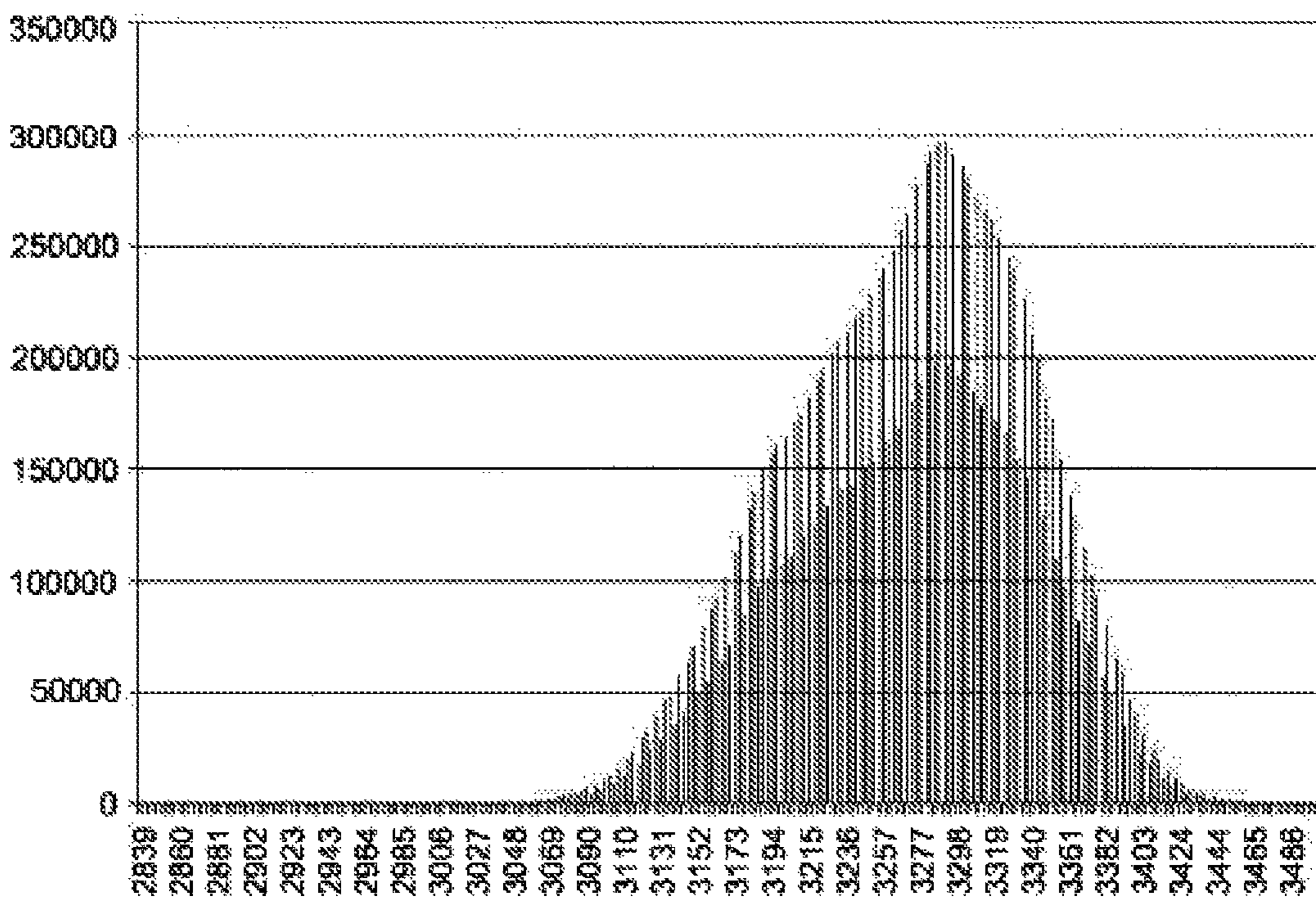


FIG. 6B



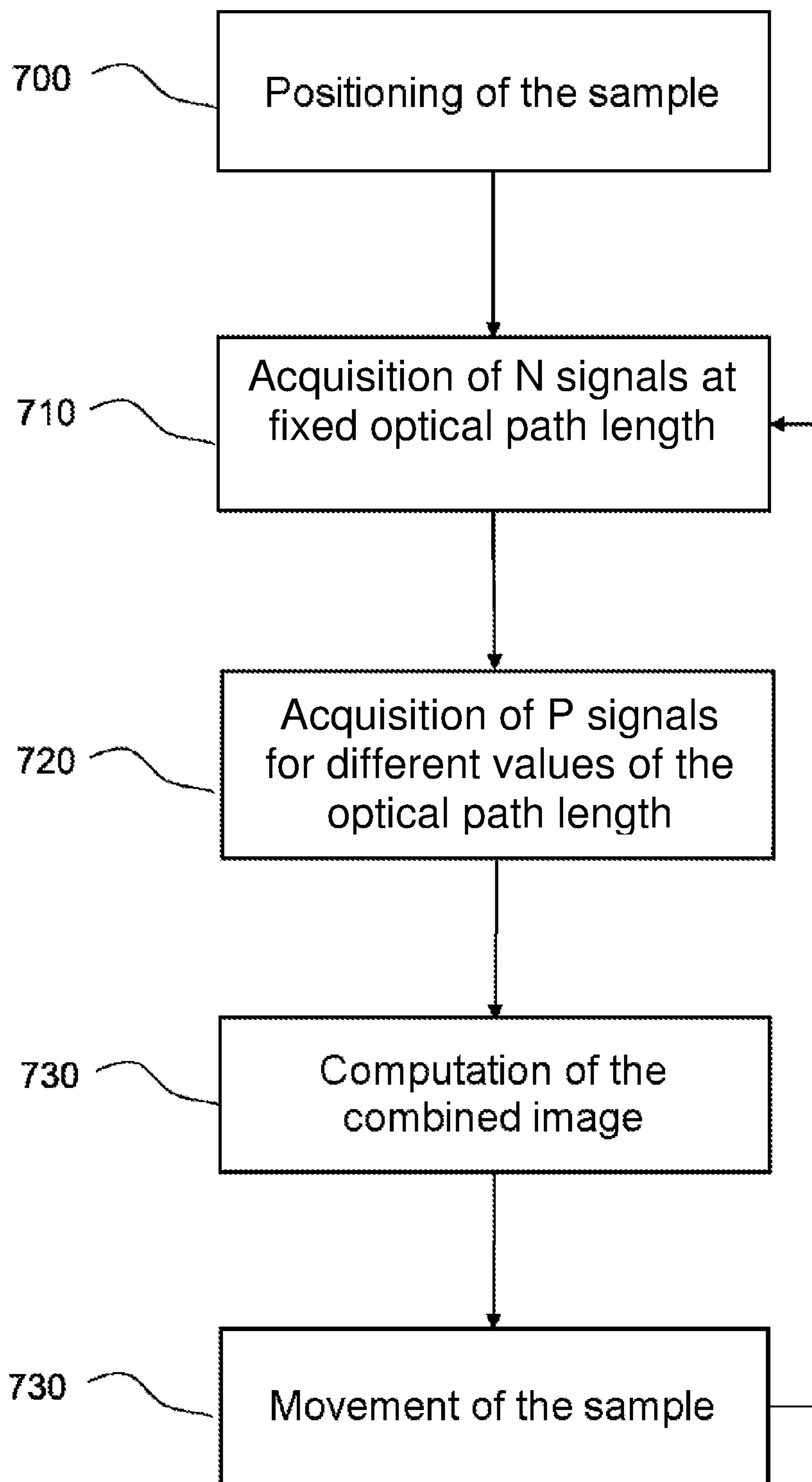


FIG.7



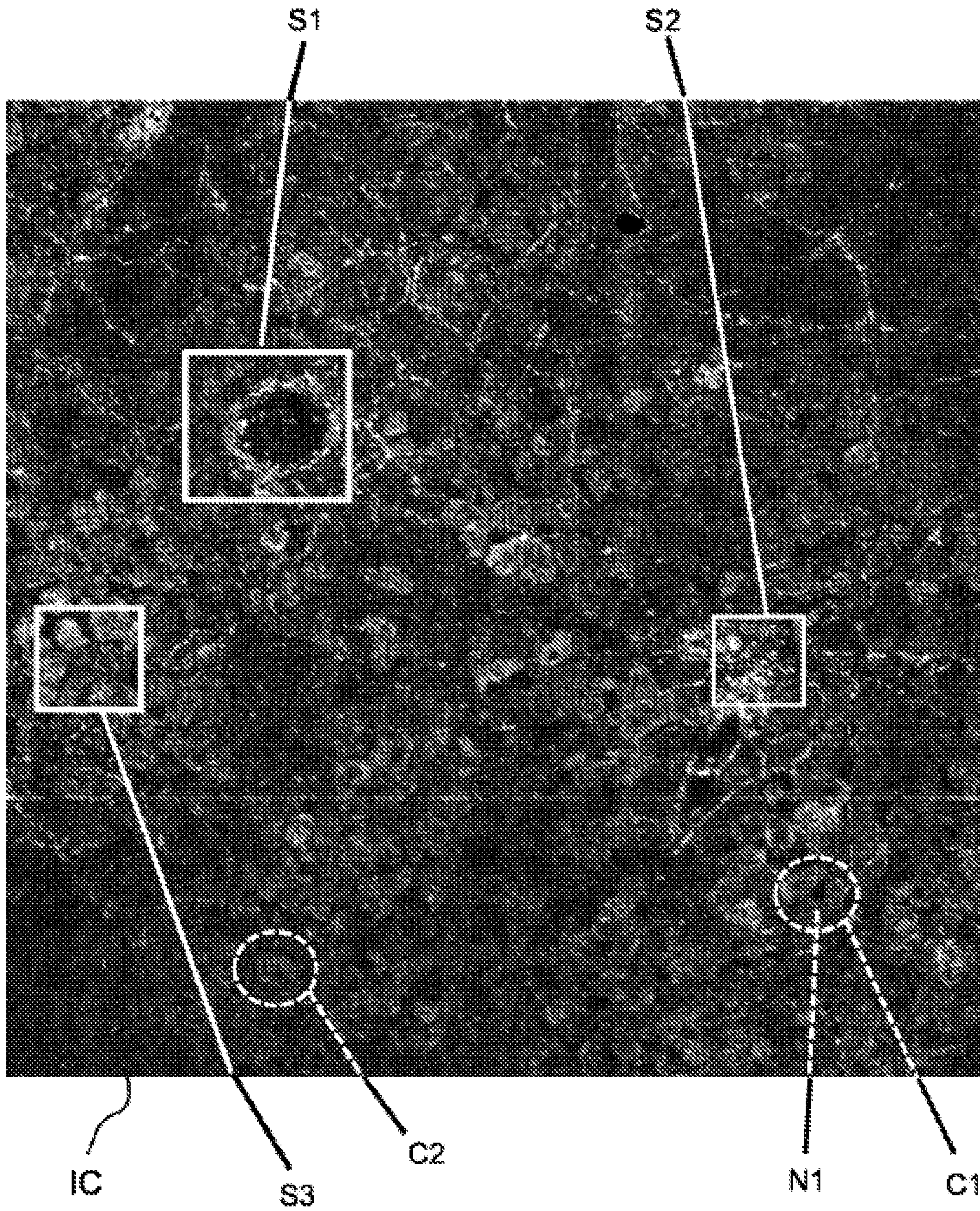


FIG.8



## METHOD AND SYSTEM FOR FULL-FIELD INTERFERENCE MICROSCOPY IMAGING

### TECHNICAL FIELD

The present description relates to a full-field interference microscopy imaging method and system. It is applicable in particular to cellular and intracellular imaging.

### STATE OF THE ART

The technique of image acquisition by incoherent light full-field interference microscopy, known by the name full-field OCT (OCT being the acronym for “Optical Coherence Tomography”), is a non-invasive, non-destructive and endogenous method which is very powerful for acquiring images of biological tissues.

The full-field OCT imaging technique is for example described in the article “Full-field optical coherence tomography” by A. Dubois and C. Boccara, taken from the work “Optical Coherence Tomography—Technology and Applications”—Wolfgang Drexler—James G. Fujimoto—Editors—Springer 2009. The full-field OCT imaging technique is also described in the French patent application FR2817030.

The full-field OCT imaging technique is based on the use of the light backscattered by a sample when it is illuminated by a light source with low coherence length, and in particular the use of the light backscattered by the microscopic cell and tissue structures in the case of a biological sample. This technique exploits the low coherence of the light source to isolate the light backscattered by a virtual slice depthwise in the sample. The use of an interferometer makes it possible to generate, by an interference phenomenon, an interference signal representative of the light originating selectively from a given slice of the sample, and to eliminate the light originating from the rest of the sample.

The full-field OCT imaging technique makes it possible to obtain images in three dimensions with a typical resolution of the order of 1  $\mu\text{m}$ , which is greater than the resolutions of the order of 10  $\mu\text{m}$  likely to be obtained with other conventional OCT techniques such as OCT in the spectral domain (known by the acronym “Fourier-Domain OCT” or “spectral domain OCT”).

With such a resolution, it is possible to view most of the tissue structures of blood vessels, of their walls, the collagen, the adipocytes, etc. This technique thus makes it possible to view microstructures of diverse biological tissues such as the brain, the breast, the skin, the liver, etc., as is described for example in the article “*Large Field, High Resolution Full-Field Optical Coherence Tomography: A Pre-clinical Study of Human Breast Tissue and Cancer Assessment*”, O. Assayag et al., Technology in Cancer Research and Treatment Volume 13, No. 5, October 2014. Furthermore, this technique proves to be particularly fast: it is thus possible to generate, using a full-field OCT microscope, an image representative of a depthwise slice whose surface is several  $\text{cm}^2$  in just a few minutes.

FIG. 1 is an example of an image obtained by the full-field OCT technique for a tissue sample of a liver (optical biopsy performed on a rat liver) on which collagen fibers can in particular be seen. The image, reference IA, corresponds to a sample zone of 800  $\mu\text{m}$  by 800  $\mu\text{m}$  (micron) size. This figure illustrates the possibilities of this technique for revealing and viewing very fine biological structures.

It has been shown that the full-field OCT image acquisition technique can potentially be used for medical applica-

tions such as diagnosing cancer, since the viewing of the microstructures and of the tissue architecture makes it possible, in a great number of cases, to distinguish a healthy tissue from cancerous tissue in a few minutes, in a non-destructive manner. A full-field OCT image during the time of the operation makes it possible, for example, to produce a first level diagnosis, even optimize the surgical action in the case, for example, of cancers, to possibly avoid a repeat operation in the case where the histological analysis would reveal the presence of tumor cells just a few days after excision.

Furthermore, the full-field OCT technique allows image acquisition in-vivo, possibly in-situ with, for example, endoscopes, which reinforces the relevance of the technique in the medical field.

However, although the full-field OCT technique offers a theoretical spatial resolution that is sufficient to generate images of biological tissues to a cellular and intracellular scale (of the order of 1  $\mu\text{m}$  in the three dimensions), the applicants have shown that, in practice, it does not always make it possible to clearly distinguish the cells, nor even nuclei or internal structures of cells (membrane, nucleus, cytoplasm in particular) because of an insufficient contrast in the images obtained.

For example, in the example of FIG. 1, the backscattered signal originating from collagen fibers exhibits, because of the high refractive index of the lipid and protein structures, a high intensity. By contrast, the backscattered signal originating from internal structures of the cells is of very low intensity, approximately 400 times lower than that of the collagen fibers. The difference of intensity of the signals backscattered by the different structures of the cell and of the tissue explains how, despite the excellent spatial resolution of the full-field OCT technology, this technology does not make it possible to acquire, with a sufficient dynamic range, the signals of low intensity.

Other microscopy techniques used in anatomical pathology, for their part, make it possible to view, by means of a microscope, cells and cell structures on a tissue sample: these techniques consist in taking a tissue sample in a zone to be analyzed, then preparing a slide of tissue that can be observed under a conventional microscope. The preparation consists in placing the tissue in paraffin, in cutting very thin slices thereof—of the order of 3 to 5  $\mu\text{m}$  of thickness—and in applying contrast or coloring agents to these slices that make it possible to raise the contrast of the cell structures and thus facilitate the viewing thereof by the anatomical pathology doctor. Typically, a combination of hematoxylin and eosin is applied to these slices, in order to color the support structures and the nuclei of the cells specifically. Other colorations can also be applied to allow for certain structures to be viewed even more specifically. The resulting tissue slice is then placed on a microscope slide and observed at various enlargements by the anatomical pathologist. The macroscopic examination with low enlargement and the microscopic examination with strong enlargement make it possible to observe the tissue architecture and the cell details present on the tissue sample. A set of reading criteria makes it possible to perform a diagnosis of the cancer, with, for example, the examination of the stromal reaction resulting from the tumoral invasion or even the examination of the nucleus/cytoplasm ratio at the individual cell level.

These microscopy techniques do however present several drawbacks. Firstly, these techniques are destructive, since, contrary to the full-field OCT technique which makes it possible to obtain an image of a virtual slice of the tissue



sample, these techniques necessitate preparing a physical slice of the sample as described. The tissue will thus no longer be usable for other types of analyses. Also, these techniques rely on a complex preparation of the sample, including the physical handling thereof during multiple steps (placing in paraffin, cutting, marking) as well as the use of exogenous contrast agents such as specific colorants of cellular structures; the final quality of the sample thus prepared, and consequently the quality of the corresponding diagnosis, is therefore dependent on the operator. Lastly, these techniques are not suited to a peroperative use, because, given the different steps to prepare the tissue, several days are generally needed before being able to proceed with an observation of the tissue.

There therefore emerges a need for an imaging technique for the acquisition of images of a sample, notably of a biological tissue, which at least partly remedies the limitations and/or drawbacks of the prior art.

### SUMMARY

According to a first aspect, the present description relates to a system for full-field interference microscopy imaging of a diffusing three-dimensional sample comprising:

an interference device comprising an object arm intended to receive the sample and a reference arm on which a reflection surface is arranged, the interference device being adapted to produce, when the sample is disposed on the object arm of the interference device, at each point of an imaging field, an interference between a reference wave obtained by reflection of incident light waves on an elementary surface of the reflection surface corresponding to said point of the imaging field and an object wave obtained by backscattering of incident light waves by a voxel of a slice of the sample at a given depth, said voxel corresponding to said point of the imaging field,

an acquisition device adapted to acquire, with fixed optical path difference between the object arm and the reference arm, a temporal succession of N two-dimensional interferometric signals resulting from the interferences produced at each point of the imaging field, a processing unit configured to compute an image representative of temporal variations of intensity between said N two-dimensional interferometric signals.

The imaging field is limited in its dimensions for example by a useful detection surface of the acquisition device or by a field diaphragm of the imaging system. Thus, according to one example, the imaging field at the level of the slice of the sample (or coherence slice) is the zone conjugate with the useful surface of the acquisition device (or with the field diaphragm). A "point" of the imaging field is defined in its dimensions by the dimensions of an elementary detector of the acquisition device.

An elementary surface of the reflection surface corresponding to a point of the imaging field represents, in the present description, an elementary surface defined on the reflection surface and whose position corresponds to the position of the point of the imaging field defined in relation to a two-dimensional coordinate system associated with the acquisition device.

A voxel corresponding to a point of the imaging field represents, in the present description, an elementary volume defined in the coherence slice and whose position in the coherence slice corresponds to the position of the point of the imaging field defined in relation to a two-dimensional coordinate system associated with the acquisition device. A

wave backscattered by a voxel is representative of the amplitude of the coherent sum of the waves backscattered by all of the elementary diffusing structures present in this voxel.

The imaging system thus described makes it possible to obtain an image of the sample representative of temporal variations of intensity between the interferometric signals with fixed optical path difference, and for which the applicants have shown that it made it possible to access information not perceptible by means of the images obtained according to the full-field OCT techniques according to the prior art. Fixed optical path difference should be understood here to mean that the variation of optical path between the reference arm and the object arm is constant; for example, there is no relative variation of the position of the sample in relation to the reflection surface.

The advantages associated with this imaging technique in the field of cellular or intracellular imaging devolve notably from the observations made by the applicants as to the activity occurring at the cellular or intracellular level, and more specifically of the different movements causing the temporal variations of intensity between the interferometric signals: flow or circulation of liquids and also intracellular movements of different organites (or "organelles") such as cytoskeleton, cell nuclei, mitochondria, lysosomes, etc.

According to one or more embodiments of the imaging system, the processing unit is configured to compute the image by computing, for each pixel of given position in this image, a pixel value as a function of a value of a parameter representative of the temporal variations of intensity of the N two-dimensional interferometric signals acquired at a point of corresponding position in a two-dimensional coordinate system associated with the acquisition device.

The extraction of a parameter representative of the temporal fluctuations or variations, over a time period, of the intensities of the interferometric signals acquired at a given point, makes it possible to reveal, in the computed image, tissue and cell regions of the sample in which movements occur.

According to one or more embodiments, this parameter is a parameter representative of the temporal dispersion of the intensities of the N two-dimensional interferometric signals considered. Such a parameter is for example the standard deviation of the statistical distribution of the intensities. In this way, a global measurement is performed that is representative of the temporal dispersion of the light intensities backscattered at a given point of the biological tissue.

A representation in image form of the values obtained for this parameter makes it possible to reveal and view the tissue regions where movements occur.

According to one or more embodiments of the imaging system, a pixel of the image exhibits at least one component, defined in relation to a colorimetric representation space, whose value is a function of the value of the chosen parameter. For example, in the case of an image in gray levels, the zones of the sample which are animated by a significant movement and for which the value of this parameter is therefore high, emerge in such images with a high gray level. On the other hand, the parts for which no movement is detected and exhibiting a zero parameter value, will exhibit a very low gray level.

This image acquisition method is particularly interesting in the case of tissues exhibiting a wide variety of structures and therefore of diffusers, for which the dynamic range of the backscattered signal is very wide.

According to one or more embodiments of the imaging system:



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the acquisition device is configured to also acquire P two-dimensional interferometric signals for the sample slice for different values of the optical path difference between the two arms of the interference device, the processing unit is configured to compute an image, called combined image, from the intensities of the P two-dimensional interferometric signals and said temporal variations of intensity.

Compared to a tomographic image obtained by a full-field OCT imaging method according to the prior art, the combined image constitutes a representation of the sample that is more comprehensive and more rich in information on the different structures present in the sample because it combines, on the one hand, the information likely to be obtained by a full-field OCT imaging method according to the prior art, such information being representative of the intensities of the signals backscattered by the different voxels and, on the other hand, the information representative of the temporal variations of intensity of the signals backscattered by the different voxels.

According to one embodiment of the imaging system, the processing unit is configured to compute the combined image by computing, for a pixel of given position in the combined image, a pixel value as a function, on the one hand, of the intensities of the P two-dimensional interferometric signals acquired at a point of corresponding position in a two-dimensional coordinate system associated with the acquisition device and, on the other hand, of the temporal variations of intensity of the N two-dimensional interferometric signals acquired at a point of corresponding position in a two-dimensional coordinate system associated with the acquisition device.

The different embodiments of the imaging system according to the present description can be combined with one another.

According to a second aspect, the present description relates to a method for full-field interference microscopy imaging of a diffusing three-dimensional sample comprising:

the placement of the sample on an object arm of an interference device,

the production, by means of the interference device, for each point of an imaging field, of an interference between a reference wave obtained by reflection of incident light waves on an elementary surface of a reflection surface of a reference arm of the interference device, said elementary surface corresponding to the point of the imaging field, and an object wave obtained by backscattering of the incident wave by a voxel of a slice of the sample at a given depth, said voxel corresponding to said point of the imaging field,

the acquisition, with fixed optical path difference between the object arm and the reference arm, of a temporal succession of N two-dimensional interferometric signals resulting from the interferences produced at each point of the imaging field,

the computation of an image representative of temporal variations of intensity between the N two-dimensional interferometric signals.

According to one embodiment of the imaging method, the step of computation of the image comprises the computation, for each pixel of given position in the image, of a pixel value as a function of a value of a parameter representative of the temporal variations of intensity of the N two-dimensional interferometric signals acquired at a point of corresponding position in a two-dimensional coordinate system associated with the acquisition device.

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According to a particular embodiment, this parameter is representative of the temporal dispersion of the intensities of the N two-dimensional interferometric signals acquired.

According to one embodiment of the imaging method, a pixel of the image exhibits at least one component, defined in relation to a colorimetric representation space, whose value is a function of the value of the chosen parameter.

According to one embodiment, the imaging method further comprises:

the acquisition of at least P two-dimensional interferometric signals for the sample slice for different values of the optical path difference between the two arms of the interference device,

the computation of an image, called combined image, from the intensities of the P two-dimensional interferometric signals and said temporal variations of intensity.

According to one embodiment of the imaging method, the computation of the combined image comprises, for each pixel of given position of the combined image, the computation of a pixel value as a function, on the one hand, of the intensities of the P two-dimensional interferometric signals acquired at a point of corresponding position in a two-dimensional coordinate system associated with the acquisition device and, on the other hand, of the temporal variations of intensity of the N two-dimensional interferometric signals acquired at a point of corresponding position in a two-dimensional coordinate system associated with the acquisition device.

The advantages stated for the imaging system can be transposed to the imaging method according to the second aspect of the present description.

The different embodiments of the imaging method according to the second aspect of the present description can be combined with one another.

Different features and embodiments of the various aspects of the present description can also be combined with one another.

## BRIEF DESCRIPTION OF THE FIGURES

Other advantages and features of the imaging technique presented hereinabove will become apparent on reading the following detailed description, with reference to the figures in which:

FIG. 1 (already described) is an example of an image, called FFOCT image, of a sample of biological tissue (rat liver) having collagen fibers produced by using a full-field OCT imaging method according to the prior art; such a method will hereinafter be called FFOCT imaging method;

FIG. 2 is a theoretical block diagram of an embodiment of an imaging system according to the present description;

FIG. 3 is a flow diagram of an embodiment of an imaging method according to the present description; this method will hereinafter be called DC-FFOCT imaging method,

FIG. 4 is an example of an image, called DC-FFOCT image, of the same sample as that represented in FIG. 1, at the same point, in the same coherence slice, obtained by using a DC-FFOCT imaging method according to the present description;

FIGS. 5A and 5B represent curves of variation of intensity of the signals acquired in an example of DC-FFOCT imaging method at 2 points of the sample of which an image is presented in FIG. 4, the 2 points forming part of zones respectively exhibiting an absence of intracellular movement and a presence of an intracellular movement;



FIGS. 6A and 6B represent the statistical distribution of the signaling intensities whose variation curves are represented respectively in FIGS. 5A and 5B;

FIG. 7 is a flow diagram of an embodiment of an imaging method for the generation of a combined image; such a method will hereinafter be called CDC-FFOCT (for “Combined Dynamic Contrast FFOCT”) imaging method;

FIG. 8 is an example of an image, called CDC-FFOCT image, resulting from a CDC-FFOCT imaging method as described with reference to FIG. 7 and representing the same sample as that represented in FIGS. 1 and 4, at the same point and in the same coherence slice.

## DETAILED DESCRIPTION

### Imaging System

An embodiment of an imaging system **20** suitable for implementing a method for imaging a three-dimensional sample according to the present description is schematically represented in FIG. 2.

The imaging system **20** comprises an interference device **200**, an acquisition device **208** and at least one processing unit **220**.

The interference device **200** is adapted to produce optical interferences between, on the one hand, reference waves obtained by reflection of the light emitted by a light source **201**, spatially incoherent and of low coherence length, by each elementary surface of a reflection surface **205** of a reference arm of the interference device and, on the other hand, of the object waves obtained by backscattering of the light emitted by the same source by each voxel of a slice of a sample **206** depthwise in the sample, the sample **206** being disposed on an object arm of the interference device, said voxel and said elementary surface corresponding to the same point of the imaging field.

The light source **201** is a source that is incoherent or of low coherence length (in practice, in a range from 1 to 20 micrometers), for example a halogen lamp or an LED. According to one or more exemplary embodiments, the light source **201** can form part of the imaging system **20**, as in the example of FIG. 2, or can be an elemental external to the imaging system, the imaging system being adapted to work with light waves emitted by the source.

The acquisition device **208** allows the acquisition of at least one two-dimensional interferometric signal resulting from the interferences between the reference waves and the object waves.

The processing unit **220** is configured to execute at least one step of processing of at least one two-dimensional interferometric signal acquired by the acquisition device **208** and/or at least one step of image generation in accordance with at least one of the imaging methods according to the present description, in order to generate at least one image of the sample slice.

In one embodiment, the processing unit **220** is a computing device comprising a first memory CM1 (not represented) for the storage of digital images, a second memory CM2 (not represented) for the storage of program instructions and a data processor, capable of executing program instructions stored in this second memory CM2, in particular to control the execution of at least one step of processing of at least one two-dimensional interferometric signal acquired by the acquisition device **208** and/or of at least one step of image computation in accordance with at least one of the imaging methods according to the present description.

The processing unit can also be produced in integrated circuit form, comprising electronic components suitable for implementing the function or functions described in this document for the processing unit. The processing unit **220** can also be implemented by one or more physically distinct devices.

The acquisition device **208** is for example an image sensor, of CCD (Charge-Coupled Device) or CMOS (Complementarity metal-oxide-semiconductor) camera type. This acquisition device is capable of acquiring images at a high rate, for example with a frequency of 100 Hz. Depending on the dynamics of the sample studied, and more specifically the dynamics of the movements within the sample, it will be possible to use the cameras operating from a few Hz up to several KHz.

According to one embodiment, the interferometer **200** comprises a beam-splitter element **202**, for example a non-polarizing splitter cube, making it possible to form two arms. In one of the arms, which will hereinafter be called “reference arm” there is the reflection surface **205**, flat, for example a mirror. The other arm, which will hereinafter be called “object arm”, is intended to receive, in operation, the three-dimensional diffusing sample **206**, of a slice of which there is a desire to produce a tomographic image at at least one depth according to one of the methods of the present description.

In the example of FIG. 2, the interferometer is of Linnik interferometer type and comprises two identical microscope lenses **203**, **204** arranged on each of the arms. The reflection surface **205** is thus located at the focus of the lens **204** of the reference arm and the sample **206** is intended to be positioned at the focus of the lens **203** of the object arm. Other types of interferometers can be envisaged for the implementation of the methods according to the present description, and in particular interferometers of Michelson, Mirau, Fizeau and other such types.

At the output of the interferometer **200** there is an optic **207**, for example an achromatic doublet, whose focal length is adapted to allow a suitable sampling of the sample **206** by the acquisition device **208**, and which makes it possible to conjugate the planes situated at the foci of the two lenses in one and the same plane at the output of the interference device. The acquisition device **208** is placed in the latter plane in order to acquire the interference signals produced by the interference device. In order to not limit the resolution permitted by the microscope lenses **203** and **204**, the choice of the focal length of the optic **207** will be in line with the Shannon criterion. The focal length of the optic **207** is for example a few hundreds of millimeters, typically 300 mm.

Glass plates **209**, **210** are if necessary provided on each of the arms to compensate the dispersion.

Since the light source **201** has a low coherence length, interferences between the light reflected by the reflection surface **205** (reference wave) and that backscattered by the sample **206** occur only when the optical paths in the two arms are equal, to within the coherence length. Thus, interferences occur between the reference wave and the light backscattered by each voxel of a slice situated in a plane at right angles to the optical axis of the object arm, at a given depth of the sample, called coherence slice, a voxel being an elementary volume defined in the coherence slice. The light backscattered by each voxel is representative of the amplitude of the coherent sum of the waves backscattered by all of the diffusing elementary structures present in this voxel.

The interferometric signals resulting from the optical interferences between the reference waves and the waves backscattered by the different voxels are acquired in parallel



at an instant  $t$  by the acquisition device **208**. The result thereof is an interferometric image  $S$  corresponding to the state of interference at a given instant  $t$  of the coherence slice. An interferometric image element or image pixel situated at a given position  $(x,y)$ , defined in relation to a two-dimensional coordinate system associated with the acquisition device **208**, exhibits a value  $S(x,y,t)$  which corresponds to the intensity of the interferometric signal, acquired at the instant  $t$  at the position  $(x,y)$ , resulting from the interference between the wave backscattered by the voxel of corresponding position in the sample and the reference wave reflected by an elementary surface of the reflection surface **205** of the reference arm of corresponding position.

More specifically, the light intensity  $S(x, y, t)$  of the interferometric signal acquired by the acquisition device **208** at the position  $(x,y)$  and at the instant  $t$ , can be expressed in the form:

$$S(x, y, t) = \frac{S_0}{4} (RA(x, y) + RB(x, y, t) + R_{ref} + 2\sqrt{RB(x, y, t)R_{ref}} \cos\phi(x, y, t)) \quad (1)$$

in which:

$S_0$  is the light intensity of the incident wave at the input of the interferometer;

$RA(x,y)$  is an equivalent reflection coefficient, proportional to the intensity of the light backscattered by the sample which does not interfere, that is to say the light backscattered by structures of the sample situated outside of the coherence slice;

$RB(x,y,t)$  is an equivalent reflection coefficient, proportional to the intensity of the light backscattered by a voxel of the sample **206** in the coherence slice, at a given position  $(x, y)$  and at a given instant  $t$ ;

$R_{ref}$  is the reflection coefficient of the reference surface (reflection surface **205**);

$\phi(x, y, t)$  is the relative phase between the reference wave and the wave backscattered by the voxel of the sample at the position  $(x, y)$  and at the instant  $t$ .

The processing unit **220** is configured to generate an image of the sample **206** from at least one two-dimensional interferometric signal obtained by the acquisition device **208**.

Different methods for using this imaging system and for generating images from two-dimensional interferometric signals produced by this imaging system are described in more detail hereinbelow.

#### DC-FFOCT Imaging Method

The main steps of an embodiment of an imaging method according to the present description, called DC-FFOCT (for “Dynamic Contrast FFOCT”), are described with reference to FIG. 3. This method is applied in particular to intracellular imaging and can be implemented by the imaging system **20**.

In an initial step **300**, the DC-FFOCT imaging method is initialized and a sample **206** is placed in the object arm of the interference device **200** at a position making it possible to analyze a first sample slice. This first slice is the current slice for the first execution of the steps **310** to **330** described hereinbelow.

In the step **310**, a temporal succession of  $N$  two-dimensional interferometric signals of the current slice of the sample **206** is acquired by the acquisition device **208** with

fixed optical path difference between the object arm and the reference arm. In particular, no variation of the optical path difference is produced in this case, unlike what is done for a full-field OCT imaging method according to the prior art.

According to one embodiment, the optical path difference is maintained fixed by keeping both the reflection surface in the reference arm and the sample **206** in the object arm of the interference device **200** at a fixed position. In the absence of variation of the optical path difference, the analysis of the interference signals obtained for a sample slice makes it possible to obtain a two-dimensional interferometric signal clearly revealing the movements internal to the sample **206** for the structures present in this slice of the sample.

The period of acquisition of the succession of two-dimensional interferometric signals and/or the acquisition frequency (and therefore the number of two-dimensional interferometric signals) can be chosen in a way suited to the characteristics of the cellular or intracellular movements to be revealed. For example, the more rapid these movements are, the higher the acquisition frequency will be chosen to be so as to meaningfully sample the different intensities of the interferometric signal at a given point. On the other hand, for slower movements, a lower frequency and a longer acquisition period may prove more suitable. The number  $N$  is chosen typically between  $10^2$  and  $10^4$ , for example around 1000, for an acquisition frequency of between 100 and 1000 images per second, typically 100 images per second, i.e. one acquisition over a period of between 0.1 and 100 seconds, typically ten or so seconds. These parameters can vary according to the applications targeted: as a function of the nature of the cells, of the different pathologies for which the images are to be generated or of the wavelength used. With the cameras working from a few Hz up to a kHz, a sampling is obtained that is suited to the different applications.

The acquisition of the  $N$  two-dimensional interferometric signals (step **310**) results in  $N$  interferometric images  $SN_i(x,y)$ , in which  $SN_i(x,y)$  is the intensity of the signal acquired at the position  $(x,y)$  by the detection device,  $i$  being an integer number varying from 1 to  $N$  and  $(x,y)$  representing the position, in relation to a two-dimensional coordinate system associated with the acquisition device **208**. These interferometric images are stored in the first memory **CM1** that can be accessed by the image processing unit **220** or else are transmitted to the processing unit **220**.

In accordance with the equation (1) above, each light intensity  $SN_i(x,y)$  of the interferometric signal acquired at the position  $(x,y)$  at the instant  $t=t_i$  takes the form:

$$SN_i(x, y) = \frac{S_0}{4} (RA(x, y) + RB_i(x, y) + R_{ref} + 2\sqrt{RB_i(x, y)R_{ref}} \cos\phi_i(x, y)) \quad (2)$$

in which  $S_0$ ,  $RA(x,y)$ ,  $R_{ref}$  were defined in the equation (1) above,  $RB_i(x,y)$  is the reflection coefficient proportional to the light intensity backscattered at the instant  $t_i$  by a voxel of the sample **206** at a given position  $(x, y)$  in the coherence slice, and  $\phi_i(x, y)$  is the relative phase between the reference wave and the wave backscattered by the voxel of the sample at the position  $(x, y)$  at the instant  $t_i$ .

In practice, the applicants have shown that movements of the diffusers along an axis parallel to the optical axis of the object arm (“vertical” movement) result in variations of the relative phase  $\phi(x, y)$  between the reference wave and the backscattered wave whereas movements of the diffusers in



a plane at right angles to the optical axis of the object arm (“horizontal” movement) result in variations of the reflection coefficient  $RB(x,y)$  proportional to the backscattered light intensity. In practice, the fluctuations of intensity between the light intensities  $SN_i(x,y)$  result from both “horizontal” and “vertical” movements.

The processing unit **220** of the imaging system is then used to compute an image, called dynamic contrast image or DC-FFOCT image, representative of temporal variations of intensity between these  $N$  two-dimensional interferometric signals acquired by the acquisition device **208**. The computation of this dynamic contrast image comprises the steps **320** and **330** hereinbelow.

In the step **320**, for each possible position  $(x,y)$  for a pixel in an interferometric image acquired by the acquisition device **208**, a value is computed for at least one parameter  $VN(x,y)$  representative of the temporal variations of intensity of the two-dimensional interferometric signals  $SN_i(x,y)$  acquired at a corresponding position  $(x,y)$  in the step **310** for the current slice of the sample **206**.

The temporal variations of intensities of the two-dimensional interferometric signals  $SN_i(x,y)$  are due in particular to the temporal variations of the coefficient  $RB_i(x,y)$  and of the relative phase  $\phi_i(x,y)$ , for example because of the movements of diffusers, such as certain intracellular elements in the case of a cell, within a voxel, the other terms being substantially constant over the period of acquisition of the  $N$  interferometric signals.

In one embodiment, the parameter  $VN(x,y)$  is representative of the characteristics of the statistical distribution of the intensities of the two-dimensional interferometric signals acquired, in particular of the temporal dispersion of these intensities.

This parameter  $VN(x,y)$  is for example a function of the standard deviation of the intensities  $SN_i(x,y)$ ,  $i$  varying from 1 to  $N$  in this succession of  $N$  interferometric images. For example:

$$VN(x,y) = \sqrt{\frac{1}{N} \sum_{i=1}^N (SN_i(x,y) - MN(x,y))^2} \quad (3)$$

$$\text{with } MN(x,y) = \frac{1}{N} \sum_{i=1}^N SN_i(x,y) \quad (4)$$

This parameter  $VN(x,y)$  can also be a mean value of standard deviations computed respectively over different subsets of the  $N$  interferometric images.

Other statistical or mathematical parameters making it possible to characterize the amplitude of the temporal variations of intensity of the pixels and/or the dispersion of these intensities can be used, such as the variance (standard deviation squared), the interquartile deviation, the extent (difference between its highest value and its lowest value), etc.

The parameter  $VN(x,y)$  can also be obtained by various space-time signal processing methods: self-correlation coefficient of the temporal signals  $SN_i(x,y)$  obtained for each pixel  $(x,y)$ , or breakdown into singular values for example.

In the step **330**, a dynamic contrast image  $IB$  or DC-FFOCT image is computed that is representative of temporal variations of intensity between the  $N$  two-dimensional interferometric signals of the current slice of the sample **206**.

In one embodiment, each pixel  $IB(x,y)$  of the image  $IB$ , situated at a given position  $(x,y)$ , represents the value computed for this given position for the parameter chosen in

the step **320**. For example, a pixel of the image  $IB$  which is situated at a given position  $(x,y)$  and/or at least one component of this pixel, defined in relation to a colorimetric representation space, exhibits a value which is a function of the value computed for the parameter concerned for the corresponding position  $(x,y)$  from the intensities  $SN_i(x,y)$ , for  $i=1$  to  $N$ , of the  $N$  interferometric signals acquired in the step **310**.

For example, when the colorimetric representation space used for the image  $IB$  is a representation on gray levels, the value of the pixel  $IB(x,y)$  can be equal to or a function of the value  $VN(x,y)$  to within a scaling factor so as, for example, to obtain a gray level coded on a given number of bits.

According to another example, when the colorimetric representation space used for the image  $IB$  is a representation according to the RGB (Red, Green, Blue) colorimetric representation space, at least one of the components  $R$ ,  $G$  or  $B$  of the pixel  $IB(x,y)$  of position  $(x,y)$  in the image  $IB$  will be equal to or a function of  $VN(x,y)$  to within a scaling factor so as, for example, to obtain a colorimetric component coded on a given number of bits.

According to yet another example, when the colorimetric representation space used for the image  $IB$  is a representation according to the YUV (luminance/chrominance) colorimetric representation space, at least one of the components  $Y$ ,  $U$  or  $V$  of the pixel  $IB(x,y)$  of position  $(x,y)$  in the image  $IB$  will be equal to or a function of  $VN(x,y)$  to within a scaling factor so as, for example, to obtain a component coded on a given number of bits.

The dynamic image  $IB$  thus produced can then be displayed on a display screen **230** linked to the image processing unit.

FIG. 4 represents an example of a dynamic image  $IB$  produced by the DC-FFOCT imaging method for the same coherence slice of the sample as that represented in the image of FIG. 1. The pixels of this image are coded on gray levels. The image corresponds to a sample zone of  $800 \mu\text{m}$  by  $800 \mu\text{m}$  size extracted from a rat liver. In this image, fine structures are distinguished, in particular individual cells, which were not present in FIG. 1. Not only are hepatic cells  $C1$ ,  $C2$  distinguished (see the zones outlined in dotted lines), but also their nucleus (darker circular zone  $N1$  in the middle of the hepatic cell  $C1$ ). Such details were not present or not visible in the image of FIG. 1, obtained by a full-field OCT imaging technique according to the prior art for the same sample slice.

The example of FIG. 4 illustrates in particular the benefit of the method according to the present description for cellular and intracellular imaging and in particular highlights the significant enhancement of the contrast of the cellular and intracellular structures.

On a microscopic scale, in particular at the cellular or intracellular level, various movements occur: flow or circulation of liquids, but also intracellular movements of different organites (or “organelles”) such as cell nuclei, mitochondria, lysosomes, etc. This is true not only for the in-vivo tissues of living humans and animals or plants, but also for the samples freshly taken from the living humans and animals or plants. For example, the complete death (apoptosis) of a human cell of a sample only occurs 24 to 48 hours after the taking of this sample from the living person. During this time period, the continued life and movement can be detected, particularly in the cells.

The biological tissues also exhibit optical backscattering properties such that approximately a few thousandths to a few millionths of the power of the incident light beam are backscattered by these tissues because of the presence in



these tissues of diverse structures, hereinafter in the description called “diffusers”, whose size is smaller than or close to that of the wavelength of the light beam.

The movements of the fine structures present in the tissue like the intracellular structures produce phase differences of the backscattered beam and, through the interferences, temporal variations of the light intensity of the signal acquired by the interferential microscope. These temporal variations of intensities can be detected for a given time period, by acquisition at a given frequency, of a succession of two-dimensional interferometric signals of the same sample slice. By proceeding with an acquisition of two-dimensional interferometric signals with fixed optical path difference between the object arm and the reference arm, the applicants have shown that it is possible to sample, at the desired rate, the two-dimensional interferometric signals resulting from the interference states occurring in succession in a slice of the sample and proceed with an analysis of the temporal variations of intensity between these two-dimensional interferometric signals in order to produce an image of the tissue regions of the sample in which the movements which are the source of these temporal variations of intensity occur.

By way of examples, FIGS. 5A and 5B illustrate the temporal variations of the intensities of the two-dimensional interferometric signals acquired by the acquisition device 208 for two positions respectively corresponding to two positions  $(x_1, y_1)$  and  $(x_2, y_2)$  in the image IB represented in FIG. 4. FIGS. 6A and 6B represent the statistical distributions of the signal intensities for which the variation curves are represented respectively in FIGS. 5A and 5B. For a first position  $(x_1, y_1)$  in a two-dimensional coordinate system associated with the acquisition device 208, it can be seen in FIG. 5A that the intensities of the signals recorded on 12 bits vary overall between 3445 and 3450 (i.e. a variation of approximately 5 units) with a temporal and average distribution that is substantially stable over time. At this position  $(x_1, y_1)$ , which is for example a position situated in the zone S2 represented in FIG. 4, centered on collagen fibers, the absence of movement is deduced. For a second position  $(x_2, y_2)$ , which is for example a position situated in the zone S3 represented in FIG. 4, inside a hepatic cell, it can be seen in FIG. 5B that the intensities of the signals vary overall between 3231 and 3239 (i.e. a variation of approximately 9 units) with a greater temporal dispersion and a substantially variable average over time. FIG. 5B is representative, on the other hand, of a position  $(x_2, y_2)$  where there is an intracellular movement. By comparison to FIGS. 6A and 6B, it is possible to observe a greater temporal dispersion in the distribution, represented in FIG. 6B, of the intensities of the signal, represented in FIG. 5B, in relation to the temporal dispersion, represented in FIG. 6A, of the intensities of the signal, represented in FIG. 5A.

After having computed a DC-FFOCT image for a given coherence slice, a determination is made in the step 340 as to whether to acquire a DC-FFOCT image for another sample slice. In the affirmative, the position of the sample along the optical axis of the object arm is modified during the step 340 and the steps 310 to 330 are repeated for this second slice of the sample which becomes the current slice. Otherwise, the method is terminated.

A three-dimensional image of the sample 206 can thus be obtained by varying the position of the sample 206, and therefore the position depthwise in the sample 206 of the coherence slice for which the optical paths in the two arms of the interference device are equal, and by repeating the interferometric signal acquisition and image generation procedure for this coherence slice.

The steps 320 and 330 of generation of the dynamic image IB can also be executed after the step 340, subject to retaining in memory, for each sample slice, the N interferometric images acquired in the step 310.

The image acquisition steps 310 for a sample slice can also be executed in parallel to the steps 320 and 330 of generation of the dynamic image for a preceding slice of the sample 206.

#### CDC-FFOCT Imaging Method

The steps of an embodiment of the method for generating a combined image, also called CDC-FFOCT (for “Combined Dynamic Contrast FFOCT”) imaging method are described with reference to FIGS. 7 and 8.

The CDC-FFOCT imaging method is implemented by using the imaging system 20 and a modulation device 211, suitable for varying the relative phase between the reference wave and the backscattered wave. The modulation device makes it possible, according to one example, to generate a relative movement of the reflection surface in relation to the sample, for example by means of a piezoelectric plate for the movement of the reflection surface; the result thereof is a variation of the optical path difference between the object arm and the reference arm of the interference device 200, as will be described in more detail hereinbelow.

In an initial step 700, the CDC-FFOCT imaging method is initialized and a sample 206 is placed in the object arm of the interference device 200 at a position that makes it possible to analyze a first sample slice. This first slice is the current slice for the first execution of the steps 710 to 730 described hereinbelow.

The step 710 is identical to the step 310 described for the DC-FFOCT imaging method. In particular, a temporal succession of N two-dimensional interferometric signals of the current slice of the sample 206 is acquired by the acquisition device 208 with fixed optical path difference between the object arm and the reference arm.

In the step 720, according to an exemplary embodiment, a succession of P two-dimensional interferometric signals of the current slice of the sample 206 is acquired by the acquisition device 208 for different values of the optical path difference between the object arm and the reference arm of the interference device 200. The variation of the optical path difference is effected for example by movement, parallel to the optical axis of the reference arm, of the reference surface 205 by means of the piezoelectric plate 211, for example a movement according to a sinusoidal function resulting in a modulation of the optical path difference. A synchronization with the image capture performed by the acquisition device 208 can then make it possible to record interferometric images for predefined values of the phase of the two-dimensional interferometric signals.

From the acquisition of the interferometric signals in the step 720, there result P interferometric images, denoted  $SP_j$ , in which  $SP_j(x,y)$  is the intensity of the interferometric signal acquired at the position  $(x,y)$  and for a relative phase between the reference wave and the backscattered wave  $\varphi=\varphi_j$ , j being an integer number varying from 1 to P,  $P \geq 2$ , and  $(x,y)$  representing the position in relation to a two-dimensional coordinate system associated with the acquisition device 208. These interferometric images  $SP_j(x,y)$  are stored in a memory CM1 accessible by the processing unit 220 or else are transmitted to the processing unit 220.

The light intensity  $SP_j(x,y)$  of the interference signals acquired by the acquisition device 208 for the position  $(x,y)$  and for a relative phase  $\varphi=\varphi_j$  can be written in the form:



$$SP_j(x, y) = \frac{S_0}{4} (RA(x, y) + RB(x, y) + R_{ref} + 2\sqrt{RB(x, y)R_{ref}} \cos\phi_j(x, y)) \quad (5)$$

in which  $S_0$ ,  $RA(x,y)$ ,  $R_{ref}$  have been defined in the equation (1) above,  $RB(x, y)$  is an equivalent reflection coefficient proportional to the light intensity backscattered by a voxel of the sample **206** at a given position  $(x, y)$  in the coherence slice and  $\phi_j(x, y)$  is the relative phase between the reference wave and the wave backscattered by the voxel of the sample at the position  $(x, y)$ .

Each pixel value of the resulting full-field OCT image, corresponding to the coefficient  $RB(x, y)$ , is obtained by a combination of the intensity values  $SP_j(x,y)$  according to known methods.

In the case of a variation of the phase resulting from a temporal modulation of the relative movement of the reflection surface in relation to the sample for example, the phase  $\phi_j(x, y)$  corresponds to the relative phase between the reference wave and the wave backscattered by the voxel of the sample at the position  $(x, y)$  and at an instant  $t=t_j$ . The applicants have demonstrated however that the temporal fluctuations of the coefficient  $RB(x, y)$  are very low in relation to the coefficient  $RB(x, y)$  sought (typically a factor 1000). It is therefore possible to reasonably approximate  $RB(x, y)$  as constant as a function of time.

In one embodiment,  $P=2$  interferometric images are acquired, for two values of the optical path difference corresponding to a phase shift of  $\pi$  between the relative phases  $\phi_j(x, y)$ . Thus, a first interferometric image is recorded for a first value of the relative phase  $\phi_1(x, y)$  and a second interferometric image is recorded for a second value of the relative phase,  $\phi_2(x, y)=\phi_1(x, y)+\pi$  for example. Each pixel value of the resulting full-field OCT image, corresponding to the coefficient  $RB(x, y)$ , is obtained by computing the difference between two intensity values  $SP_1(x,y)$  and  $SP_2(x,y)$  obtained respectively for two relative phase values  $\phi_1(x, y)$  and  $\phi_2(x, y)$  then by calculating therefrom the absolute value of the difference between the two values  $SP_1(x,y)$  and  $SP_2(x,y)$ .

The technique described previously of acquisition of  $P$  two-dimensional interferometric signals corresponds to what is used in an FFOCT imaging method according to the prior art. Any other method for computing  $RB(x, y)$  in accordance with a full-field FFOCT imaging method according to the prior art can be used.

In particular, it is possible to proceed in a known manner with a modulation of the optical path difference and with a detection synchronized by the detection device in order to acquire  $P$  interferometric signals for  $P$  values of the optical path difference to determine the FFOCT image. It is also possible to integrate, the interferometric signal over fractions of modulation period, for example over 4 periods, to obtain the amplitude and the phase of the interferometric signal.

It is also possible to do the acquisition at the same time (at the same instant) of several interferometric images corresponding to different relative phase values, for example two relative phase values separated by  $\pi$ , for example by spatial splitting of 2 beams phase-shifted by  $\pi$ .

In the step **730**, the processing unit **220** of the imaging system is then used to compute an image, called combined image or CDC-FFOCT image, representative also of the

temporal variations of intensity between the  $N$  two-dimensional interferometric signals acquired by the acquisition device **208** in the step **710**.

The combined image  $IC$  is computed from, on the one hand, the intensities of the  $P$  two-dimensional interferometric signals acquired by the acquisition device **208** in the step **720** and, on the other hand, from the temporal variations of intensities between the  $N$  two-dimensional interferometric signals acquired by the acquisition device **208** in the step **710**.

In a first embodiment, a first intermediate image  $IB$ , or dynamic contrast image, is computed from the temporal variations of intensity between the  $N$  two-dimensional interferometric signals acquired by the acquisition device **208** in the step **710** and a second intermediate image  $IA$ , called tomographic image, is computed from the intensities of the  $P$  two-dimensional interferometric signals acquired by the acquisition device **208** in the step **720**. The combined image  $IC$  is then computed by pixel-to-pixel combination of these two intermediate images  $IA$  and  $IB$ . Pixel-to-pixel combination should be understood here to mean that a pixel  $IA(x,y)$  of position  $(x,y)$  in the image  $IA$  is combined with the pixel  $IB(x,y)$  of the same position  $(x,y)$  in the image  $IB$ .

In a second embodiment, no intermediate image is generated and the combined image  $IC$  is computed by directly computing the pixels of the combined image from the intensities of the interferometric signals acquired. In particular, for a pixel of given position  $(x,y)$  in the combined image  $IC$ , a pixel value  $IC(x,y)$  is computed as a function, on the one hand, of the intensities of the  $P$  two-dimensional interferometric signals acquired at a point of corresponding position in a two-dimensional coordinate system associated with the acquisition device and, on the other hand, from the temporal variations of intensity of the  $N$  two-dimensional interferometric signals acquired at a point of corresponding position in a two-dimensional coordinate system associated with the acquisition device.

In the first embodiment, as in the second, a pixel value  $IC(x,y)$  of given position  $(x,y)$  in the combined image is computed by combination of two pixel values  $IA(x,y)$  and  $IB(x,y)$ , in which:

the value  $IA(x,y)$  is computed from the intensities of the  $P$  two-dimensional interferometric signals acquired for different values of the optical path difference for the position  $(x,y)$  and represents the coefficient  $RB(x,y)$  proportional to the intensity of the wave backscattered by a given voxel of the coherence slice of the sample, and

the value  $IB(x,y)$  is computed from the intensities of the  $N$  two-dimensional interferometric signals acquired with fixed optical path difference and represents the temporal variations of the intensities determined between the  $N$  two-dimensional interferometric signals acquired at the point of corresponding position in a coordinate system associated with the acquisition device.

The value  $IB(x,y)$  is computed according to the method described for the step **330** for example. In particular, the value  $IB(x,y)$  can be computed as a function of the value of at least one parameter  $VN(x,y)$  representative of the temporal variations of the intensities  $SN_i(x,y)$  between the  $N$  interferometric signals.

It is assumed here, for the combination of pixel values, that the acquisition of the signals performed in the steps **710** and **720** has been performed such that the signal  $SN_i(x,y)$  acquired for the position  $(x,y)$  in the step **710** originates from the same voxel of the sample **206** as the signal  $SP_j(x,y)$



acquired for the position (x,y) in the step 720: this will be the case if neither the sample 206, nor the acquisition device 208 is moved during the execution of the steps 710 and 720 and the acquisition device 208 acquires images of the same resolution in the steps 710 and 720 and by using the same two-dimensional coordinate system.

The combination of pixel value  $IA(x,y)$  and  $IB(x,y)$  is performed such that the information contained respectively in each of the images  $IA(x,y)$  and  $IB(x,y)$  for a given voxel of the sample 206 is present in the resulting combined image IC. This combination can be performed such that a user viewing the resulting image IC is able to discriminate the structures revealed in the image IA (tomographic image) from the fluctuations of structures revealed in the image IB (dynamic contrast image).

In particular, the value  $IC(x,y)$  of a pixel of the combined image which is situated at a given position (x,y) in the image IC is function of the value  $IA(x,y)$  of the pixel situated at this same position (x,y) in the tomographic image IA and a function of the value computed for this position (x,y) for the parameter used in the step 720. The pixel  $IC(x,y)$  can exhibit a component defined in relation to a colorimetric representation space.

Thus, a user will be able to view, for a given point of the sample 206, an image containing both the information originating from a known full-field interference microscopy imaging in incoherent light and the information on the movements of the fine structures of the sample 206 revealed by the DC-FFOCT imaging method according to the present description.

Various methods for combining these values  $IA(x,y)$  and  $IB(x,y)$  are possible and the methods described hereinbelow are given as nonlimiting examples.

A first combination method consists in assigning, for each position (x,y) in a zone of the combined image, the pixel value  $IA(x,y)$  to a first colorimetric component of a pixel  $IC(x,y)$  of the image IC and the pixel value  $IB(x,y)$  to a second colorimetric component of this pixel  $IC(x,y)$ . For example, by using a representation according to the RGB colorimetric representation space, the component R of the pixel  $IC(x,y)$  of position (x,y) in the image IC will be equal to the pixel value  $IA(x,y)$  and the component B (Blue) of the pixel  $IC(x,y)$  of position (x,y) in the image IC will be equal to the pixel value  $IB(x,y)$  or vice versa.

A second combination method consists in defining two look-up tables TA, TB (referred to by the acronym LUT), a first look-up table TA for the pixel values  $IA(x,y)$  and a second look-up table TB for the pixel values  $IB(x,y)$ . Each look-up table TA, respectively TB, serves to compute, for each possible position (x,y) in a zone of the combined image, a pixel value  $TA(x,y)$ , respectively  $TB(x,y)$ , resulting from the transformation of the pixel value  $IA(x,y)$ , respectively  $IB(x,y)$  by the look-up table TA, respectively TB. The value of a pixel  $IC(x,y)$  at a position (x,y) in the image IC is then obtained by a function of combination of the pixel value  $TA(x,y)$  and of the pixel value  $TB(x,y)$ . This combination function is for example a "logic OR" of the pixel values  $TA(x,y)$  and  $TB(x,y)$ , or else a function of addition of these two pixels or of computation of the mean or sum value weighted by weighting coefficients.

The resulting image IC can be a color image or a gray level image depending on the combination method chosen.

In the step 730, the combined image IC thus generated can then be displayed on a display screen 230 linked to the image processing unit.

FIG. 8 represents an example of an image IC resulting from the combination of the image IA represented in FIG. 1

with the image IB represented in FIG. 4. The method used for the combination is for example the second method as described above. Each of these images represents one and the same zone and one and the same sample slice of 800  $\mu\text{m}$  by 800  $\mu\text{m}$  size as the zone and the slice imaged in FIGS. IA and IB (biological tissue of rat liver). By comparison of the image IA of FIG. 1 with the image IC of FIG. 8, it clearly emerges that the image IC is much richer in fine structures than the image IA but nevertheless includes the information of the image IA of FIG. 1. These fine structures of the image IC correspond in reality to fluctuations detected in the slice of the sample concerned: the fine structures of the image of FIG. 8 are those originating from the image IB (FIG. 4) which has been combined with the image IA (FIG. 1).

As an example, in the image IA of FIG. 1, it is possible to distinguish numerous collagen fibers, a zone S2 of collagen fiber concentration and a large capillary in the zone S1, but individual cells are not distinguished. By contrast, in the image IB of FIG. 4, individual cells are distinguished in the zones C1, C2 or S3, but not the collagen fibers of the zone S2 or the large capillary of the zone S1. In the image IC, the collagen fibers (zone S2), the large capillary (zone S1) and the individual cells (zones C1, C2 or S3) are all distinguished.

By virtue of the combined image IC, a user can have a single image containing a great deal of visual information on the structures present in the slice of the sample, just as on the fluctuations, representative of the movements and cellular or intracellular activities of this same sample slice.

Once a CDC-FFOCT combined image is computed for a given coherence slice of the sample, a determination is made, in the step 740, as to whether to generate a CDC-FFOCT combined image for another sample slice. In the affirmative, the position of the sample along the optical axis of the object arm is modified in the step 740 and the steps 710 to 730 are repeated for this second slice of sample which becomes the current slice. Otherwise, the method is terminated.

A three-dimensional image of the sample 206 can thus be obtained by varying the position of the sample 206, and therefore the position depthwise in the sample 206 of the coherence slice for which the optical paths are the same in both arms of the interference device, and by repeating the interferometric signal acquisition and image generation procedure for this coherence slice.

The DC-FFOCT and CDC-FFOCT imaging methods described hereinabove are applied in particular to the acquisition of images of cells or of intracellular images, and can prove particularly useful for diagnosing cancer where the examination of the cellular details proves necessary. In particular, the dynamic origin of the contrast generated by the DC-FFOCT and CDC-FFOCT imaging methods can make it possible, in addition to viewing cellular details, to view the cellular activity and distinguish the metabolic status of a cell (overactivity, dead cell, necrosis).

They can also be applied generally in all situations where the different voxels of the coherence slice of the sample to be analyzed exhibit movements, activities, functions, mechanisms or physical-chemical properties leading to a temporal variation of light intensity reflected by these voxels. They are also applicable to any samples, whether these samples are biological or not.

The invention claimed is:

1. A system for full-field interference microscopy imaging of a diffusing three-dimensional sample comprising:



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an interference device comprising:

an object arm configured to receive the sample, and  
 a reference arm on which a reflection surface is  
 arranged, the interference device being adapted to  
 produce, when the sample is disposed on the object  
 arm of the interference device, at each point of an  
 imaging field, an interference between a reference  
 wave obtained by reflection of incident light waves  
 on an elementary surface of the reflection surface  
 corresponding to said point of the imaging field, and  
 an object wave obtained by backscattering of inci-  
 dent light waves by a voxel of a slice of the sample  
 at a given depth, said voxel corresponding to said  
 point of the imaging field;

an acquisition device configured to acquire, with fixed  
 optical path difference between the object arm and the  
 reference arm, a temporal succession of N two-dimen-  
 sional interferometric signals resulting from the inter-  
 ferences produced at each point of the imaging field;  
 and

a processing unit configured to compute an image repre-  
 sentative of temporal variations of intensity between  
 said N two-dimensional interferometric signals,

wherein the acquisition device is further configured to  
 acquire P two-dimensional interferometric signals for  
 the sample slice for different values of the optical path  
 difference between the object arm and the reference  
 arm of the interference device, and

wherein the processing unit is configured to compute a  
 combined image from the intensities of the P two-  
 dimensional interferometric signals and said temporal  
 variations of intensity.

**2.** The imaging system as claimed in claim 1, wherein the  
 processing unit is further configured to compute said image  
 by computing, for each pixel of given position in said image,  
 a pixel value as a function of a value of a parameter  
 representative of the temporal variations of intensity of the  
 N two-dimensional interferometric signals acquired at a  
 point of corresponding position in a two-dimensional coor-  
 dinate system associated with the acquisition device.

**3.** The imaging system as claimed in claim 2, wherein said  
 parameter is representative of the temporal dispersion of the  
 intensities of the N two-dimensional interferometric signals.

**4.** The imaging system as claimed in claim 2, wherein said  
 pixel exhibits at least one component, defined in relation to  
 a colorimetric representation space, whose value is a func-  
 tion of the value of said parameter.

**5.** The imaging system as claimed in claim 1, wherein the  
 processing unit is further configured to compute said com-  
 bined image by computing, for a pixel of given position in  
 said combined image, a pixel value as a function, on the one  
 hand, of the intensities of the P two-dimensional interfero-  
 metric signals acquired at a point of corresponding position  
 in a two-dimensional coordinate system associated with the  
 acquisition device and, on the other hand, of the temporal  
 variations of intensity of the N two-dimensional interfero-  
 metric signals acquired at a point of corresponding position  
 in a two-dimensional coordinate system associated with the  
 acquisition device.

**6.** A method for full-field interference microscopy imag-  
 ing of a diffusing three-dimensional sample comprising:

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placement of the sample on an object arm of an interfer-  
 ence device;

production, by means of the interference device, for each  
 point of an imaging field, of an interference between a  
 reference wave obtained by reflection of incident light  
 waves on an elementary surface of a reflection surface  
 of a reference arm of the interference device, said  
 elementary surface corresponding to said point of the  
 imaging field, and an object wave obtained by back-  
 scattering of the incident wave by a voxel of a slice of  
 the sample at a given depth, said voxel corresponding  
 to said point of the imaging field;

acquisition, with fixed optical path difference between the  
 object arm and the reference arm, of a temporal suc-  
 cession of N two-dimensional interferometric signals  
 resulting from the interferences for each point of the  
 imaging field;

computation of an image representative of temporal varia-  
 tions of intensity between the N two-dimensional inter-  
 ferometric signals;

acquisition of at least P two-dimensional interferometric  
 signals for the sample slice for different values of the  
 optical path difference between the object arm and the  
 reference arm of the interference device; and

computation of a combined image from the intensities of  
 the at least P two-dimensional interferometric signals  
 and said temporal variations of intensity.

**7.** The imaging method as claimed in claim 6, wherein the  
 step of computation of the image comprises the computa-  
 tion, for each pixel of given position in said image, of a pixel  
 value as a function of a value of a parameter representative  
 of the temporal variations of intensity of the N two-dimen-  
 sional interferometric signals acquired at a point of corre-  
 sponding position in a two-dimensional coordinate system  
 associated with the acquisition device.

**8.** The imaging method as claimed in claim 7, wherein  
 said parameter is representative of the temporal dispersion  
 of said intensities of the N two-dimensional interferometric  
 signals.

**9.** The imaging method as claimed in claim 7, wherein  
 said pixel exhibits at least one component, defined in rela-  
 tion to a colorimetric representation space, whose value is a  
 function of the value of said parameter.

**10.** The imaging method as claimed in claim 6, wherein  
 the computation of the combined image comprises, for each  
 pixel of given position of the combined image, the compu-  
 tation of a pixel value as a function, on the one hand, of the  
 intensities of the P two-dimensional interferometric signals  
 acquired at a point of corresponding position in a two-  
 dimensional coordinate system associated with the acquisi-  
 tion device and, on the other hand, of the temporal varia-  
 tions of intensity of the N two-dimensional interferometric  
 signals acquired at a point of corresponding position in a two-  
 dimensional coordinate system associated with the acquisi-  
 tion device.

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